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# *A PERICENTRIC INVERSION IN GASTERIA RESULTING IN APPARENT ISO-CHROMOSOMES AT MEIOSIS*

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In a study of chromosome behavior in a plant of *Gasteria* it was found[1] that an anomalous type of disjunction occurs at meiosis in one of the seven tetrad chromosomes. The chromosome involved (designated the *SML* chromosome) has a sub-median centromere. The dyads resulting from the *SML* tetrad disjoin at anaphase I in three ways:

1. *Type I.*—The two dyads, one at each pole of the spindle figure, are composed of two long and two short arms. These arms may be joined with one another as normal chromatids, or similar arms may be joined to form either long or short metacentric chromatids.

2. *Type II.*—The dyad at one pole has one short and three long arms, the dyad at the opposite pole has one long and three short arms.

3. *Type III.*—One dyad has four short arms, the other, four long arms.

The separation of the dyad chromatids at anaphase II gives rise to five different kinds of quartets with respect to the types of chromosomes derived from the original *SML* tetrad and their arrangement. These quartet types are as follows:

1. Type A (derived from Type I) has four normal *SML* chromosomes arranged in the regular manner at the poles.

2. Type B (from Type II) contains two normal chromosomes and both kinds of metacentric chromosomes, with a metacentric chromosome separating from a normal in each anaphase figure.

3. Type C (from Type III) contains only metacentric chromosomes, with the two long chromosomes separating from one another in one anaphase figure, the two short chromosomes separating from one another in the other anaphase figure.

4. Type D (from Type I) also has only metacentric chromosomes, but the two dissimilar kinds are separating from one another in each anaphase figure.

5. Type E (from Type I) is similar to Type B in having three kinds of chromosomes, but the two normal chromosomes are separating from one another in one anaphase figure, while the two dissimilar metacentric chromosomes are separating from one another in the sister anaphase figure.

The other six tetrad chromosomes of the complement behave in a regular manner at both meiotic divisions. Consequently, three kinds of microspores are produced at meiosis, all having the same chromosome number, but differing in the kind of chromosome received from the division of the aberrant tetrad. About half the microspores receive a normal chromosome from this tetrad; the other half receive metacentric chromosomes, the two complementary types being equally frequent. Only two of these microspore types, the one containing a normal haploid complement and the one having the long-armed metacentric chromosome, are able to develop and pass through the first post-meiotic mitosis, and it is clear that the development of the latter type is delayed as compared with the normal. Microspores containing a short-armed metacentric chromosome have never been observed at the microspore mitosis.

The peculiar chromosome behavior at meiosis was originally explained as resulting from a postulated aberrant type of centromere division at metaphase I of meiosis. However, it now appears unnecessary to invoke such aberrant centromere behavior in order to explain the observations. A more detailed study of meiotic prophase stages and a further analysis of the types and frequencies of the various quartets at anaphase II have shown that the results can be explained on the basis of crossing-over in a heterozygous pericentric inversion in the *SML* tetrad, a possibility suggested to the writer by Dr. Barbara McClintock.

It has not been possible to make a complete analysis of prophase pairing in the *SML* chromosome. However, synaptic figures have been found at pachytene indicating the presence of an inversion of some length, although it is not possible to be certain that the *SML* chromosome is involved. It seems probable that the figures represent a pericentric inversion since bridge configurations are very infrequent at anaphases I and II, suggesting that paracentric inversions are at most few in number and quite short. An examination of tetrad configurations has been made at diplotene and diakinesis. It is very difficult to fix these so-called diffuse stages properly in *Gasteria*, but reasonably good results have been obtained by using an osmic acid fixative, staining by the Feulgen method, and counterstaining with fast green to reveal the nucleolus.[2] Since the nuclelous is attached to the long arm of the *SML* tetrad, the latter can be easily located. Some of the configurations of this tetrad chromosome strongly suggest by the position of chiasmata and the relationships of the long and short arms that crossing-over has occurred within an inversion including the centromere.

The analysis of quartets at AII is particularly instructive, since the various types and their frequencies can all be logically explained as a result of single or double crossing-over within a pericentric inversion whose length is approximately equal on each side of the centromere. The synapsis of a normal chromosome and one with the inversion would give rise to an inversion loop configuration with the centromere in approximately the middle of the loop. Crossing-over within this inversion produces the previously described types of quartets as follows:

1. Type A results from (*a*) no crossing-over within the inversion, (*b*) two-strand double crossing-over on one side of the centromeres, either to the right or to the left, or (*c*) two-strand double crossing-over, the two crossovers being on opposite sides of the centromere.

2. Type B results from (*a*) a single crossover on either side of the centromeres, or (*b*) a three-strand double on one side, either to the right or to the left.

3. Type C results from a four-strand double on one side of the centromeres, either to the right or to the left.

4. Type D results from a four-strand double, the two crossovers being on opposite sides of the centromeres.

5. Type E results from a three-strand double, the two crossovers also being on opposite sides of the centromeres.

TABLE 1

FREQUENCIES OF THE FIVE TYPES OF QUARTETS AT AII. FOR A DESCRIPTION OF THE TYPES AND A DISCUSSION OF HOW THEY ARISE SEE TEXT

| TYPE OF QUARTET | NO. OF CELLS | PER CENT |
|---|---|---|
| A | 126 | 18.7 |
| B | 374 | 55.4 |
| C | 57 | 8.4 |
| D | 40 | 5.9 |
| E | 78 | 11.6 |
| Total | 675 | 100.0 |

The frequencies of the five types are indicated in table 1. As is expected, the types (C, D and E) which result from double crossing-over only are much less frequent than Type B, which is the only single crossover type. A further comparison can be made between Types D and E which both result from double crossing-over, the two crossovers being on opposite sides of the centromere. Type D results from a four-strand double and Type E from a three-strand double. If crossing-over involves the four chromatids at random[3, 4], E should be twice as frequent as D. It is clear from table 1 that E is indeed approximately twice as frequent as D. The possible distorting effect on this ratio of triple crossing-over (with two crossovers on one side of the centromeres and one on the opposite side) has been con-

sidered. It is found that triple crossing-over within the inversion does not affect the D:E ratio, since with random chromatid crossing-over Types B, D and E are produced, but the D:E ratio remains 1:2. Thus there is good evidence for random crossing-over of chromatid strands. The comparative frequencies of the various quartet types resulting from double crossing-over (especially Types C and D) indicate that double crossovers on one side of the centromeres are higher than double crossovers involving segments on opposite sides of the centromeres. The reason for this result is not clear, but it may mean that incomplete synapsis occurs on one side of the inversion loop in some of the pollen mother cells. Synapsis of this type should lead to an increased frequency of double crossovers on one side of the centromeres as compared with doubles involving segments on opposite sides.

The presence of chromosomes with a median centromere at anaphase II indicates that the inversion in the altered *SML* chromosome must be of approximately equal length on either side of the centromere. Otherwise, chromosomes with non-median centromeres would result from crossing-over. Furthermore, it is clear that crossing-over must occur with a very high frequency in the proximal regions of the *SML* tetrad (and observations on chiasmata at diplotene support this conclusion) in order to account for the frequencies of novel quartet types at AII. This unusual combination of circumstances gives rise to the configurations at anaphases I and II which were originally interpreted as iso-chromosomes produced by centromere misdivision at metaphase I. The present evidence indicates that the two types of metacentric chromosomes are not true iso-chromosomes in the sense of Darlington,[5] since they result from crossing-over and consequently do not have genetically identical arms. Morphologically, however, the metacentric chromosomes are indistinguishable from iso-chromosomes in metaphase and anaphase figures. Furthermore, they are also similar to iso-chromosomes in being duplication-deficiency types. The fact that the metacentric chromosomes carry certain regions of the *SML* chromosomes in duplicate and are deficient for other regions explains the previously mentioned observations made at the first post-meiotic mitosis in the developing microspores. It will be recalled that microspores carrying the short-armed metacentric chromosome (having a long deficiency) never develop as far as the pollen mitosis whereas those microspores carrying the long-armed type (having a short deficiency) do go through the pollen mitosis, but are delayed in their development as compared with microspores having a normal chromosome complement.

*Summary.*—Further cytological studies have been made of a plant of *Gasteria* which gives rise to large numbers of two types of metacentric chromosomes (in addition to the normal type) as a result of the aberrant behavior of a single one of the seven tetrad chromosomes at meiosis. The

evidence obtained from meiotic prophase configurations and from the types and frequencies of the various quartets at anaphase II indicates that these new types of chromosomes arise from crossing-over in a heterozygous pericentric inversion rather than from aberrant centromere behavior as previously suggested. The presence of metacentric chromosomes indicates that the inversion is of approximately equal length in the two arms of the altered chromosome and the frequencies of novel quartet types show that crossing-over occurs within the inversion with a high frequency. The ratio of comparable quartet types resulting from three- and four-strand double crossing-over agrees with that expected if crossing-over involves the four chromatids at random.

[1] Giles, Norman H., Jr., *Genetics*, **28**, 512–524 (1943).
[2] Hillary, B. B., *Bot. Gaz.*, **102**, 225–235 (1940).
[3] Beadle, G. W., and Emerson, S., *Genetics*, **20**, 192–206 (1935).
[4] Weinstein, A., *Ibid.*, **21**, 155–199 (1936).
[5] Darlington, C. D., *Jour. Genet.*, **39**, 351–361 (1940).

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## *STUDIES ON THE DEVELOPMENT OF THE EYE: EVIDENCE THAT THE LOBE[2], LOBE[4], LOBE[5] AND EYELESS[2] MUTANTS OF DROSOPHILA MELANOGASTER DEVELOP IN A MANNER SIMILAR TO BAR**

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*Introduction.*—Studies in the field of developmental genetics, although primarily directed toward an understanding of how the gene acts, have in many instances led toward a better understanding of the developmental mechanics of the organisms studied. This is most certainly true of *Drosophila melanogaster*, in which our knowledge of its embryology, both descriptive and experimental, has been derived from studies concerning the development of various mutant forms.

Studies on the development of the Bar mutation of *D. melanogaster* have led to a hypothesis concerning its (the mutation's) mode of development which, if capable of generalization, would add further information to our growing knowledge of the embryology of Drosophila. These studies showed that the Bar eye disc was smaller than that of wild type at all

larval stages from 36 hours after hatching, the earliest stage studied, to pupation. They demonstrated that the rate of increase in size of the Bar eye disc was the same as that of wild type and that histological differentiation proceeded at the same rate as in wild type (Steinberg 1941*a*, 1941*b* and 1943). They have also established that both a second chromosome inhibitor of Bar, and temperature bring about their effect on facet number without affecting the size of the eye disc, although it had been shown by Medvedev (1935) for Lobe,$^{c}$ glass$^{2}$, eyeless$^{2}$ and wild type, and by Steinberg and Abramowitz (1938) for double-Bar, double infra-Bar, Bar, infra-Bar and Bar$^{4}$ that the size of the eye discs of the mature larva is directly proportional to the number of facets in the imaginal eye.

The hypothesis based on these studies postulates that the reduced size of the Bar eye disc is due to the participation of fewer cells in the initial formation of the eye disc, and that this smaller size of the eye disc determines the range over which the facet number of the adult eye may vary; the variation in the facet number, once the eye disc is formed, is postulated to be due to the presence of cells labilely determined to form either head chitin or facets. The ultimate fate of these cells is dependent upon various extrinsic and intrinsic factors—such as temperature, nutrition, modifiers, etc. (Steinberg 1941*a*, 1941*b* and 1943). Reduced to its bare essentials the hypothesis postulates two main steps in the determination of the facet number of Bar, the first in the embryonic stage when the size of the eye disc and hence the possible range of variation of facet number are determined, the second in the larval stage when the fate of the labilely determined cells is fixed.

Some evidence exists which indicates that at least the first step postulated in the determination of the facet number is present in other mutant forms as well as in Bar. The evidence is derived from the data of Medvedev and of Steinberg and Abramowitz cited above which showed that a reduction in the facet number of the imaginal eye is associated with a proportional reduction in the size of the eye discs of the mature larvae. Hence all of the eight mutants studied in this respect show indications of the presence of the postulated first step in the determination of the adult facet number. There is no evidence available concerning the presence or absence of labilely determined cells in any mutant type other than Bar. This paper reports the results of experiments designed to obtain such evidence for four mutants of *D. melanogaster*.

*Materials and Methods.*—The mutants used were the alleles Lobe$^{2}$, Lobe$^{4}$ and Lobe$^{5}$ ($L^{2}$, $L^{4}$ and $L^{5}$, respectively) located at 72.0 in the second chromosome and eyeless$^{2}$ ($ey^{2}$) located in the fourth chromosome. The stocks bearing the mutants were maintained in mass cultures.

These mutants were chosen for study because previous experience had shown that the facet number of flies of each of these genotypes is extremely

variable even in isogenic stocks raised under uniform conditions, and that the facet numbers of the left and right eyes of flies of each of these mutant types are largely independent of each other. It was reasoned that this variability is due, at least in part, to an extreme sensitivity of the labilely determined cells to minor fluctuations in the environment in which the larvae develop.

If the variation in facet number from fly to fly within a given genotype is due simply to changes in the size of the eye discs, it follows that the coefficient of variability of the facet number should not be greater than that of the size of the eye discs of mature larvae of the same strain. On the other hand, if the variation in facet number from fly to fly is associated with the presence of labilely determined cells, the coefficient of variation of the facet number should exceed that for the size of the eye discs. Likewise, if the variation in facet number between the left and right eyes of a given fly is due solely to variation in the size of the left and right eye discs it follows that the left-right correlation values for the eye discs should be the same as that for the corresponding facet numbers, while, on the other hand, if the variation is due to the presence of labilely determined tissue, the left-right correlation of the eye discs should exceed that of the facets.

The experiment consisted, therefore, in counting the facet number of the left and right eyes of flies of each of the four mutant strains and of determining the areas of the left and right eye discs of mature larvae of each of the four. The facet counts were made by dissecting off the cornea of the eye, mounting it on a slide in a drop of water and then projecting the image. This method permits of great accuracy in the determination of the facet number, hence the statistics determined from these data are also accurate. The areas of the optic discs were determined by means of planimeter tracings of camera lucida drawings of eye discs newly dissected from mature larvae in a Ringer's solution (NaCl, 7.5 g.; KCl, 0.35 gms; $CaCl_2$, 0.2 g.). Because the discs are curved they must be flattened against the slide in order that the camera lucida drawings be accurate. The process of flattening, involving tapping with a needle, distorts the shape of the disc and doubtlessly introduces variations in the area of the eye discs, which may be either increases or decreases. In addition there are random errors introduced in the course of the planimeter tracings. These errors tend to increase the variability of the eye discs within a group, and precisely because they are random to decrease the left-right correlation calculated from the measurements. Consequently, the values of the statistics calculated from the eye disc measurements are shifted in such a way as to reduce their sensitivity. A further source of error is the relatively poor control of the ages of the larvae (discussed in a following paragraph). Since the eye discs increase in size as the larvae age, any variation in ages

would increase the variability of the disc size from fly to fly and again reduce the sensitivity of the statistics.

The imagos for the facet counts were raised on the standard corn meal-agar-molasses food medium, reënforced with dried brewers' yeast. The larvae used for eye disc measurements were obtained as follows: 20–30 females were permitted to lay eggs on "wafers" of food (approximately 2% Carragar plus 12.5% molasses in water), over a 24-hour-period. At the end of this period the wafers were transferred to refrigerator dishes which were half filled with the standard food medium. Larvae which had crawled up the sides of the dish preparatory to puparium formation were considered mature and were the source of the eye discs measured in the various experiments.

With one exception, all experiments were done at 25 ± 0.2°C. The exception involved the Lobe[4] flies raised for facet counts when, due to a heat wave, the temperature fluctuated between 24°C. (when ice was added to the incubator) and 28°C. It was usually closer to the latter than the former.

TABLE 1

MEAN NUMBER OF FACETS IN THE LEFT AND RIGHT EYES, AND THE MEAN AREAS OF THE CAMERA LUCIDA DRAWINGS OF THE EYE DISCS OF MATURE LARVAE OF EACH OF THE FOUR MUTANT TYPES STUDIED. THE AREAS OF THE CAMERA LUCIDA DRAWINGS ARE IN SQUARE INCHES × 100. THE LINEAR MAGNIFICATION = 114.06 ×

| | | FACETS | | | EYE DISCS | | |
|---|---|---|---|---|---|---|---|
| | | LEFT EYE | RIGHT EYE | | LEFT EYE | RIGHT EYE | |
| GENOTYPE | SEX | $\bar{X} \pm \sigma_{\bar{x}}$ | $\bar{X} \pm \sigma_{\bar{x}}$ | $N$ | $\bar{X} \pm \sigma_{\bar{x}}$ | $\bar{X} \pm \sigma_{\bar{x}}$ | $N$ |
| $L^2$ | ♂ | 106.0 ± 9.5 | 92.9 ± 9.1 | 35 | 48.9 ± 2.1 | 49.7 ± 2.0 | 25 |
| | ♀ | 134.4 ± 12.7 | 142.9 ± 10.5 | 31 | 51.5 ± 2.5 | 53.4 ± 2.2 | 24 |
| $L^4$ | ♂ | 117.6 ± 10.7 | 104.0 ± 8.9 | 26 | 48.9 ± 1.7 | 49.0 ± 1.6 | 25 |
| | ♀ | 126.9 ± 9.3 | 147.2 ± 8.6 | 37 | 56.1 ± 1.9 | 60.5 ± 2.5 | 23 |
| $L^5$ | ♂ | 135.8 ± 11.1 | 151.5 ± 9.8 | 26 | 50.2 ± 1.8 | 51.0 ± 2.6 | 25 |
| | ♀ | 153.8 ± 10.5 | 159.2 ± 11.3 | 22 | 52.6 ± 2.2 | 54.8 ± 2.0 | 25 |
| $ey^2$ | ♂ | 279.8 ± 22.4 | 339.4 ± 22.6 | 19 | 79.0 ± 3.6 | 77.2 ± 3.3 | 25 |
| | ♀ | 289.1 ± 21.7 | 290.8 ± 22.2 | 16 | 76.6 ± 5.2 | 74.2 ± 4.2 | 25 |

*Data.*—The mean facet number of the imaginal eye and the mean areas of the camera lucida drawings of the eye discs will be found in table 1. They are presented for the purpose of orientation and as a summary of the raw data from which tables 2 and 3 have been derived.

Table 2, which presents the correlation coefficients of the facet numbers in the left and right imaginal eyes and of the larval eye disc sizes, shows that in every case $r$ (correlation coefficient) is greater for the eye discs than it is for the facet numbers. Furthermore, of the eight comparisons only two, namely $L^2$ females and $L^4$ males, give a value of $P$ which exceeds 0.05. It must be emphasized, however, that even in these two cases $r$ is greater for the eye discs than for the imaginal eye. Furthermore, as pointed out in

the previous section, the estimated value for $r$ for the imaginal discs is certainly lower than its true value and therefore the observed differences between the values of $r$ for the facet numbers and the eye disc sizes are in every case lower than the true differences.

TABLE 2

CORRELATION VALUES FOR FACET NUMBERS OF THE LEFT AND RIGHT EYES AND FOR THE AREAS OF THE LEFT AND RIGHT EYE DISCS, AND THE VALUE OF $D/\sigma_D$ FOR THE COMPARISONS BETWEEN THE STATISTICS OF THE FACET NUMBERS AND THE EYE DISC AREAS

| | FACETS | | | EYE DISCS | | | |
|---|---|---|---|---|---|---|---|
| GENOTYPE | SEX | $r$ | $N$ | SEX | $r$ | $N$ | $D/\sigma_D$* |
| $L^2$ | ♂ | 0.03 | 35 | ♂ | 0.58 | 25 | 2.25 |
| | ♀ | 0.41 | 31 | ♀ | 0.67 | 24 | 1.27 |
| $L^4$ | ♂ | 0.36 | 26 | ♂ | 0.65 | 25 | 1.33 |
| | ♀ | 0.16 | 37 | ♀ | 0.68 | 23 | 2.39 |
| $L^5$ | ♂ | 0.35 | 26 | ♂ | 0.74 | 25 | 1.93 |
| | ♀ | 0.09 | 22 | ♀ | 0.66 | 25 | 2.26 |
| $ey^2$ | ♂ | −0.25 | 19 | ♂ | 0.84 | 25 | 4.42 |
| | ♀ | 0.04 | 16 | ♀ | 0.86 | 25 | 3.75 |

* Since the question asked is, "is the coefficient of correlation for the eye discs *greater than* that for the facets?" only one tail of the normal curve is used, hence $P = 0.05$ at $D/\sigma_D = 1.64$ and $P = 0.01$ at $D/\sigma_D = 2.33$ (comparisons made by converting $r$ to $Z$).

TABLE 3

COEFFICIENTS OF VARIATION FOR THE FACET COUNTS AND EYE DISC AREAS AND THE VALUES OF $D/\sigma_D$ FOR THE COMPARISONS OF THE STATISTICS OF THE CORRESPONDING FACET NUMBERS AND EYE DISC AREAS

| | | | FACETS | EYE DISCS | |
|---|---|---|---|---|---|
| GENOTYPE | SEX | | $CV \pm \sigma_{cv}$ | $CV \pm \sigma_{cv}$ | $D/\sigma_D$* |
| $L^3$ | ♂♂ | Left | 52.6 ± 6.4 | 21.1 ± 3.2 | 4.4 |
| | | Right | 57.9 ± 7.0 | 19.5 ± 3.0 | 5.1 |
| | ♀♀ | Left | 52.7 ± 6.8 | 23.7 ± 3.6 | 3.8 |
| | | Right | 40.9 ± 5.3 | 20.6 ± 3.2 | 3.3 |
| $L^5$ | ♂♂ | Left | 41.8 ± 5.9 | 18.1 ± 2.8 | 3.6 |
| | | Right | 32.9 ± 4.7 | 25.9 ± 3.8 | 1.1 |
| | ♀♀ | Left | 31.9 ± 4.9 | 20.9 ± 3.1 | 1.9 |
| | | Right | 33.4 ± 5.2 | 18.6 ± 2.8 | 2.5 |
| $L^4$ | ♂♂ | Left | 46.4 ± 6.5 | 17.2 ± 2.6 | 4.1 |
| | | Right | 43.5 ± 6.2 | 15.9 ± 2.5 | 4.2 |
| | ♀♀ | Left | 44.5 ± 5.3 | 16.6 ± 2.7 | 4.8 |
| | | Right | 35.3 ± 4.3 | 20.2 ± 3.2 | 2.8 |
| $ey^2$ | ♂♂ | Left | 34.9 ± 5.8 | 22.7 ± 3.4 | 1.8 |
| | | Right | 27.8 ± 4.9 | 21.6 ± 3.2 | 1.05 |
| | ♀♀ | Left | 31.3 ± 5.6 | 34.2 ± 5.0 | −0.37 |
| | | Right | 30.6 ± 5.5 | 28.7 ± 4.2 | 0.28 |

* Since the question asked is, "is the coefficient of variation for the eye discs *less than* that for the facets?" only one tail of the normal curve is used, hence $P = 0.05$ at $D/\sigma_D = 1.64$ and $P = 0.01$ at $D/\sigma_D = 2.33$.

Table 3 contains the data pertinent to a comparison of the coefficients of variation (*Cv*) of the larval eye discs and of the facet numbers of the imaginal eyes. Of the twelve comparisons among the Lobe alleles (i.e., facet number of the left imaginal eyes against the area of left larval eye discs, and right eyes against right eye discs of the males and females separately, making four comparisons within each genotype) only the one concerned with the right eyes and discs of the Lobe$^5$ males results in a value of $P$ greater than 0.05. This seems to be due to the relatively large coefficient of variation (*Cv*) shown by right eye discs of the Lobe$^5$ male larvae. The raw data of these eye disc measurements show one eye disc which measured 77 and another which measured 88; the next closest value to these is 61. If these two measurements are tested as possible samples of a population composed of the remaining 23 measurements it is found that neither of them fit, i.e., $P < 0.01$. Therefore, there is considerable justification for excluding these measurements from the sample. If these two values are excluded $\overline{X} = 48.3 \pm 1.64$ and $Cv = 12.2 \pm 1.8$. If this *Cv* is compared with that of the facet number $D/\sigma_D$ is found to be 4.1. It seems valid therefore to conclude that the coefficient of variation of the eye discs of the Lobe$^5$ males is smaller than that for the facet number, and therefore that the Lobe$^5$ males conform to the same pattern as the other lobe flies involved in the experiment.

The coefficients of variation derived from the *ey*$^2$ data quite clearly do not show the same relationships that those for the various lobe alleles do (table 3). In only one case, the left eyes of the *ey*$^2$ males, is $P$ less than 0.05. In the remaining cases it is considerably above 0.05. When the data for each of the four sets of eye discs are plotted in a scatter diagram, no tendency to cluster about a modal value is seen. The 25 values of each group are arranged in an almost unbroken sequence over the entire range with very little repetition of any given value. Thus in the data for the left eye discs of the males, five values occur twice, none more than twice; in the case of the right eye, one value occurs three times and all the others once each; in the data for the left eye disc of the females three values occur twice each, none more than twice; and, finally, only one value occurs twice in the data for the right eye discs of the females. Clearly the high values of the coefficients of variability shown by the eye discs of the *ey*$^2$ males and females are not due to one or two exceptional individuals as is the case with the right eye discs of the Lobe$^5$ male larvae but are a true expression of the variability of the sample. The increased variability of eyeless$^2$ eye discs relative to that of the eye discs of the Lobe alleles may be, in part or in whole, due to variation in the age of the larvae selected for dissection, for as pointed out above the age control was relatively poor. This problem will be returned to below.

*Discussion.*—The theory of development of the eye discs reviewed in the

introduction of this paper postulated in part that variation between the left and right eyes of a given individual might result from a variation in the fate of labilely determined cells in the larval eye discs. Such variation would not result in a change in the size of these discs, hence the left-right correlation should under these circumstances be greater for the larval eye discs than for the facet numbers of the imaginal eyes. The data of table 2 show quite clearly that the requirements of the hypothesis are fulfilled in each of the four mutant types studied. Hence in each of these mutant types the facet number of the imaginal eye may be varied during the larval stages by altering the path of development of labilely determined cells. These cells, in the Lobe alleles and eyeless$^2$, must be much more sensitive to minor variations in the environment than they are in Bar and wild type since in the former Margolis (1936) has shown a significant left-right correlation for the eyes of genetically Bar flies and Margolis and Robertson (1937) have shown that even marked changes in temperature during larval development fail to cause any great shift in the facet number of wild type.

These experiments taken in conjunction with those on Bar and modified Bar (Steinberg 1941*a* and 1941*b*) indicate that mutations may influence the labilely determined cells with regard to their sensitivity to environmental changes (*ey*$^2$, the Lobe alleles and Bar), and with regard to the path in development which they will take (modifier of Bar). Presumably there are mutants (perhaps among those already studied) which influence the number of labilely determined cells present in an eye disc, but no technique for detecting such effects is available at the moment.

The data in table 3, concerning the variability of the facet number of the imaginal eyes as compared with that of the corresponding eye discs, when considered in conjunction with the data of table 2, afford evidence in regard to the manner in which the range of variation of the facet number is determined in each of the specific genotypes. Variation in the facet number of the imaginal eye may arise as the result of variation in the number of cells entering into the formation of the eye disc (detectable as a variation in the size of the discs) or as a variation in the number of labilely determined cells which finally form facets (detectable by means of a higher left-right correlation for the size of the larval eye discs than for the facet number of the imaginal eye) or through some combination of the two (detectable by a combination of the above methods). The data of table 3 show that the coefficients of variation for the eye discs are in all cases significantly smaller than those for facet number. Nevertheless, the coefficients of variation for the eye discs are rather high, particularly when compared with the values derived for Bar and wild type eye discs in the author's earlier studies. However, it is entirely likely that a large part of the relatively increased variability is due to the much less accurate control of age employed in these

experiments. It will be recalled that in the present experiments age was determined simply by observing which larvae had crawled up the side of the dishes preparatory to puparium formation. In the earlier experiments, newly hatched larvae were collected over a 2-hour period and raised in 3-inch petri dishes. In the mature age group, measurements were made of the eye discs of only those larvae which were unpupated at the time when about one-half the total number of larvae in the dish had pupated. Therefore, it seems valid to conclude that in the Lobe alleles, as in Bar, the variation in facet number occurring from fly to fly is due to the variation in the number of labilely determined cells which go to form facets rather than head chitin.

The story seems to be quite different with eyeless$^2$ for here there is no difference between the coefficients of variation of the eye discs and of the facet numbers. Comparison of the data for the eye discs of eyeless$^2$ larvae with those for the eye discs of the Lobe alleles (table 3) shows that the values of the coefficients of variation of the latter are lower than those of the former. A similar comparison of the data for the facets shows that while the coefficients of variation for the facet numbers of Lobe$^2$ and Lobe$^4$ eyes are larger than those for eyeless$^2$, those for Lobe$^5$ are about the same. There is, then, a disproportionate increase in the variability of the areas of the eye discs of *ey*$^2$ larvae. There can be no doubt that the values of the coefficients of variation derived from the facet numbers of imaginal eyes are accurate since the facet counts are accurate. The same is certainly not true of the values derived from the eye disc data, for, as pointed out in the introduction, the eye discs vary in size with age and any inaccuracies in age determination would tend to increase the coefficients of variability and superimposed on this variation is the variation introduced by the technique of measurement. It is possible, therefore, that the absence of a difference between the coefficients of variation of the eye discs and of the facets is more apparent than real. Only further experiments can settle this question. If the data are correct, it would mean that the variation in facet number from fly to fly in eyeless$^2$ is dependent upon variation in the number of cells entering into the formation of the eye discs. Superimposed upon this would be the variation in the number of labilely determined cells which go to form facets. It is this variation which affects the left-right correlation coefficients. It is unlikely that variation in the number of cells entering into the formation of the eye discs would influence the value of $r$ because from the nature of the development of the eye discs it is highly probable that equal numbers of cells would enter into the formation of each disc.

*Summary.*—Previous experiments had led to the hypothesis that the facet number of the Bar eye of *Drosophila melanogaster* was determined as follows: (*a*) a reduced number of cells enter into the formation of the eye

discs thus limiting the range over which the facet number of Bar eyes can vary and (*b*) a group of labilely determined cells which may form either head chitin or facets depending upon the nature of the extrinsic and intrinsic environment determine where, within the possible range of variation of facet number, the facet number of a given eye will fall. The experiments reported in this paper were designed to test the applicability of this hypothesis to the development of Lobe[2], Lobe[4], Lobe[5] and eyeless[2]. The data show that the development of these mutants may be adequately described by the theory.

* Aided by a grant from the Penrose Fund of the American Philosophical Society.

Margolis, O. S., "Studies on the Bar Series of Drosophila III. The Facet Relations in Dorsal and Ventral Lobes of the Eye," *Genetics*, **21**, 817–831 (1936).

Margolis, O. S., and C. W. Robertson, "Studies on the Bar Series of Drosophila IV. The Temperature Effective Period for Facet Determination in the Wild Type," *Genetics*, **22**, 319–331 (1937).

Medvedev, N. N., "Genes and Development of Characters. I. The Study of the Growth of the Imaginal Discs of Eyes of the Wild Type Larvae and Three Mutants—Lobe[e], Glass[2] and Eyeless[2] in *Drosophila melanogaster*," *Zeit. ind. Abst. u. Vererb.*, **70**, 55–72 (1935).

Steinberg, A. G. A Reconsideration of the Mode of Development of the Bar Eye of *Drosophila melanogaster*. *Genetics*, **26**, 325–346 (1941*a*).

Steinberg, A. G., "Studies on Modifier of Bar. A Mutant Which Affects the Expression of the Bar Mutation of *Drosophila melanogaster*," *Genetics*, **26**, 440–451 (1941*b*).

Steinberg, A. G., "The Development of the Wild Type and Bar Eyes of *Drosophila melanogaster*," *Can. Jour. Research*, **D21**, 277–283 (1943).

Steinberg, A. G., and Abramowitz, M., "The Bar 'Locus' and the $v^+$ Reaction in *Drosophila melanogaster*," *Proc. Nat. Acad. Sci.*, **24**, 107–111 (1938).

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## *MECHANISM OF ENZYMATIC ADAPTATION IN GENETICALLY CONTROLLED YEAST POPULATIONS*

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*Introduction.*—For some time now it has been recognized that the fermentation of galactose by yeast (see Lippmann[1]) differs greatly from that of other hexoses. Armstrong[2] found that some yeasts had and others had not the power of fermenting galactose. Slator,[3] in a quantitative investigation of the same subject, was able to confirm the statement previously made, that certain yeasts which have the power of fermenting galactose, possess it only after the yeast had been acclimatized by culture in the presence of the sugar.

The nature of this acclimatization has been the subject of some investigation. The experiments of Söhngen and Coolhaas[4] would seem to indicate that the production of galactozymase parallels the formation of new cells. The results of Euler and Nilson[5] also lead to this conclusion. More recently, however, Stephenson and Yudkin[6] concluded from their experiments that the production of galactozymase in a yeast culture need not involve the formation of new cells. They interpret the phenomenon as a direct cytoplasmic reaction to the presence of the substrate (galactose) leading to the formation of a new enzyme. These authors used $CO_2$ evolution in a period when the population remained constant according to the total and viable counts.

It is clearly of some importance for the further elucidation of this acclimative or adaptive phenomenon to know whether or not new cell formation is involved in the appearance of a new physiological property in a culture. The data presented in this paper indicate that the conflict between the results and conclusions of Stephenson and Yudkin and those of previous workers cited, is more apparent than real. Both types of phenomena ("acclimatization" with and without the formation of new cells) can be observed depending upon the genetic background of the yeast strain being used.

*Difficulties in Interpretation.*—It must be recognized that microörganisms are handled in such large numbers that when a comparative biochemical investigation of microörganismic physiology is being made, over-all populational characteristics, in contradistinction to the properties of individuals, are being studied. The mechanisms available to an individual for adapting itself to an environmental change are limited by its genome and the physiological flexibility permitted by its particular degree of specialization. When, however, the adaptive ability of a population is being considered, there must be added to the physiological pliability of its members the genetic plasticity of the group in terms of the numbers and kinds of mutants it is capable of producing.

This composite nature of populational adaptability makes the interpretation of observed physiological changes in bacterial or yeast cultures difficult. In any particular case the same end-result can clearly be obtained by any one of the following: (1) the natural selection of existent mutants with the desired physiological characteristic from a heterogeneous population; (2) the presence of the substrate induces a "new"[7] enzymatic system in all the members of a homogeneous population, resulting in a gradual increase in the measured enzyme activity of the population; (3) a combination of natural selection and the action of mechanism (2) on those selected.

In most cases where so-called "training" has been observed to occur in microbic cultures, wherever a decision is at all possible, the most likely explanation seems to be that a natural selection of a variant type has taken place. Relatively few investigations (see Dubos,[8] Yudkin[9] and Karström[10]

for recent reviews) can lend much support to the hypothesis of a direct substrate-cytoplasmic interaction.

Thus far, those who have drawn the conclusion that mechanism (2) is operating have depended for the most part on demonstrating the existence of a constant total and viable count during the period when the physiological change in the population was being noted. A constant viable count alone would clearly have little critical value in deciding the question at issue. During the stationary phase of population growth new individuals must make their appearance to take the place of those that have died and natural selection could work in this period as well as in any other of the growth cycle.

However, the use of the constant *total* count involves some difficulties which may briefly be summarized as follows:

1. The failure to find "adaptation" in the complete absence of cell division would not necessarily be conclusive evidence that none exists. It is conceivable that in cultures where this "ideal" had been reached the physiological state of the cells was such that the ability to synthesize new enzymatic systems had been lost along with the ability to divide.

2. This method has the further disadvantage that it cannot distinguish between the following two situations:

(*a*) All the members of a phenotypically homogeneous population react to some component in the environment in an "adaptive" manner.

(*b*) Starting with a phenotypically heterogeneous population only those react which, due to a proper genetic constitution, already possess an adequate though latent enzymatic system. Under this condition providing the genetic variants formed a reasonably high proportion of the population (10% and up) an increased enzymatic activity with time would be observed without any concomitant cell division.

It is evident from the above discussion that the crucial point at issue in questions of this nature is the phenotypic homogeneity or heterogeneity of the starting population. And, unless in a particular case a decision is arrived at on this question, no precise or certain conclusions can be drawn, even under conditions of a constant total count, concerning the mechanism involved in accomplishing an observed change in the physiological characteristics of a population.

Since limitations of size, and sensitivity of available methods, prevent a direct examination of the individuals in a microörganismic population as a means of deciding the question of phenotypic homogeneity, recourse has been made to another method.

A cell can in general be characterized by the type of colony it produces when grown on an agar surface under standard conditions. Thus if a representative sample of a population is plated out and more than one colonial type is noted, it may be logically concluded that more than one type of in-

dividual is represented in the population. This method of examination can, under controlled conditions, yield quantitative data of a high degree of reproducibility and has been used to examine the kinetics of the appearance of morphological variants in bacterial cultures (see Shapiro, Spiegelman and Koster[11]).

*Methods and Material.*—(*A*) *Yeast Strains:* Two strains of *Saccharomyces cerevisiae* isolated in this laboratory and known as Db23B and LK2-G12 were used in the following experiments. Both strains could acquire the ability to ferment galactose if grown in its presence. Strain Db23B originated from a single ascospore and its population contains principally haploid cells and is therefore characteristically unstable. Strain LK2G12 originated from an intact 4-spored ascus in which copulation was observed to occur pairwise. Consequently it is known to be a diploid and in contrast to the Db23B is characteristically stable. For further details concerning yeast breeding and strain isolations and the implications of haploidy and diploidy for genetic stability consult Lindegren and Lindegren.[12]

(*B*) *Media:* The basic medium contains 2 g. of $KH_2PO_4$, 5 g. of peptone and 2.5 g. of dried dead yeast per liter. To this was added the desired carbohydrate. Agar plates were made by adding the requisite amount of agar to the above basic medium. It was found necessary to filter the agar medium to facilitate observation of the colonies.

(*C*) *Carbohydrates:* As sources of carbohydrate, reagent grade chemicals were used. Difco's purified galactose was further treated according to a method described by Stephenson and Yudkin[6] to remove any contaminating fermentable sugars.

(*D*) *Cell Count:* Cell counts were made by means of an haemocytometer.

(*E*) *Test Plates:* Since we are here concerned with whether or not the organisms can produce $CO_2$ from galactose, some method had to be devised which would permit the detection of this characteristic. The procedure adopted was to plate a sample of a population on an agar surface, containing 4% galactose purified as described above, and then cover it with another layer of galactose-containing agar. The production of gas cracks the agar right above the colony and thus gas producers can be distinguished easily from those which cannot evolve gas from this sugar. Figure 1 represents a typical field containing both positives and negatives.

To examine the type composition of a population growing on an ordinary glucose plate, well-isolated colonies incubated for 72 hrs. at 26°C. were used. These were removed from the agar surface and suspended in chilled galactose broth and then diluted to contain about 5000 cells per cc. as determined by direct count with an haemocytometer. Of this suspension, 0.2 cc. was then placed on a 4% galactose-4% agar surface from which all excess fluid had been allowed to drain. A sterile bent rod was then rotated over the surface to get even distribution. A 5% agar medium con-

taining 4% galactose cooled to 39° was poured over the inoculated surface. These test plates were incubated at 28°C. for at least 48 hrs. before count-

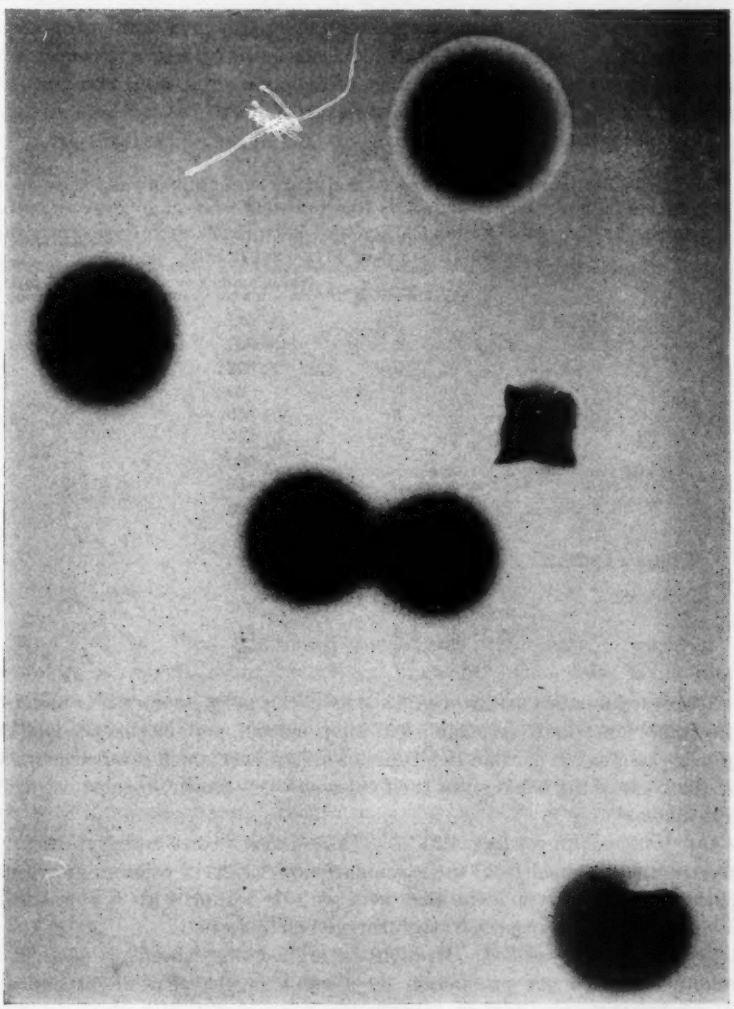

FIGURE 1

Field of a test plate in which colonies grow embedded between two layers of agar. Positives (gas producers) are easily distinguished by the typical star-shaped crack in the agar of its immediate neighborhood.

ing. Five such test plates were prepared from each suspension. Counting was done under the low power of a binocular dissecting microscope.

*Experimental Results.*—(*a*) *Data obtained from strain Db23B:* Table 1 records results of the galactose test-plate examination of the composition of 10 colonies of Db23B grown on glucose agar. It is clear that two types of individuals, at least as far as gas production from galactose is concerned, are initially present. The percentage of the population, grown on glucose, which is able to ferment galactose, varies from 2.0 to 15.0%.

TABLE 1

COMBINED TABLE SHOWING RESULTS OF EXPERIMENTS DETERMINING THE PERCENTAGE COMPOSITION OF POPULATIONS GROWN ON GLUCOSE WITH RESPECT TO THE ABILITY OF INDIVIDUALS TO PRODUCE $CO_2$ FROM GALACTOSE. EACH EXPERIMENT REPRESENTS THE COMBINED RESULTS OF FIVE TEST PLATES

| | EXPERIMENT NO. 1 | TOTAL NUMBER OF COLONIES COUNTED | AVERAGE % OF POSITIVES OBSERVED |
|---|---|---|---|
| Strain Db23B | 1 | 662 | 7 |
| | 2 | 626 | 9 |
| | 3 | 622 | 5 |
| | 4 | 625 | 15 |
| | 5 | 655 | 5 |
| | 6 | 599 | 7 |
| | 7 | 560 | 4 |
| | 8 | 685 | 2 |
| | 9 | 530 | 5 |
| | 10 | 577 | 13 |
| Strain LK2G12 | 1 | 526 | 100 |
| | 2 | 480 | 100 |
| | 3 | 692 | 100 |
| | 4 | 510 | 100 |
| | 5 | 525 | 100 |

Disregarding the quantitative aspect of the problem for the moment, the important point is established that normal populations of Db23B growing on glucose contain two types of individuals which can be classified on the basis of the behavior of their colonies when grown on galactose agar test plates.

(*b*) *Data obtained with LK2G12:* Table 1 also records some representative results obtained from the examination of LK2G12 colonies grown on glucose agar. It is evident that here we are dealing with a population homogeneous with respect to the observed characteristic.

(*c*) *Validity of method:* It might be argued that whether a particular colony exhibited gas production by a crack in the agar might depend on local characteristics of the agar in which it found itself.

Two types of experiments were undertaken to examine this question. In the first type 5% glucose rather than galactose test plates were prepared and seeded in exactly the same fashion with Db23B which was

known to ferment glucose with great ease, as determined from manometric experiments on this strain in an atmosphere of nitrogen.

If the appearance of a crack immediately above a colony did depend on local imperfections, then the same general picture should be obtained in these glucose plates as was noted in the galactose test plates. In all, 11 test plates were so prepared and 2592 colonies examined. Of these only one did not show a strong positive reaction as exhibited by the typical star-shaped crack. This latter colony when isolated and examined manometrically appeared to have the non-fermentative type of glucose oxidation.

TABLE 2

EXPERIMENTS TESTING VALIDITY OF DETECTING METHOD. PERCENTAGES ARE CALCULATED TO THE NEAREST INTEGER. EACH FIGURE REPRESENTS THE AVERAGE OF FIVE TEST PLATES

| EXPERIMENT NO. | % POSITIVES SUSPENSION 1 | % POSITIVES SUSPENSION 2 | VOLUME RATIO OF SUSP. 1:SUSP. 2 USED IN MIXTURES |
|---|---|---|---|
| 1 | 20 | 48 | 1:1 |
| 2 | 4 | 98 | 1:1 |
| 3 | 10 | 67 | 1:1 |
| 4 | 10 | 67 | 1:2 |

| EXPERIMENT NO. | COMPOSITION OF MIXTURES OBSERVED % POSITIVES | COMPOSITION OF MIXTURES CALCULATED % POSITIVES | DIFFERENCE CALCULATED − OBSERVED |
|---|---|---|---|
| 1 | 31 | 34 | 3 |
| 2 | 48 | 51 | 3 |
| 3 | 38 | 38 | 0 |
| 4 | 50 | 48 | −2 |

As a final test, the following experiment was performed: it was found, as was to be expected, that the per cent of gas-producing colonies obtained in a test plate originating from a positive colony on galactose was much higher than either from a negative one on galactose or a normal one growing on glucose. The percentage of positives obtained from such a colony varied from 75 to 99% depending on the length of incubation, a general rise with time being noted. Suspensions of equal density as determined by cell count were made of a normal colony from glucose and a positive one from a galactose plate. Five test plates were made from samples of each suspension to determine the percentage composition of the population. They were then mixed in 1:1 and 1:2 proportions and five galactose test plates made from samples of the mixtures. If the appearance of the cracks is truly a characteristic of the colony and not one of the locality in which it finds itself, it should be possible to calculate the percentages of positives to be found in the test plates of the mixtures from the percentage composition and proportions used of the two suspensions that went to make it up. The results of these experiments are recorded in table 2.

It is seen that in the first three experiments, within an average difference of 1.5 from the calculated value, the observed per cent positives in the test plates of the mixtures are the arithmetic means of the percentages of the two suspensions from which they originated. In the fourth experiment in which a 1:2 proportion was used, the deviation from the calculated value is 2%.

(*d*) *Measurements of enzyme activity:* As measured by gas evolution in nitrogen, both strains can equally well acquire the ability to ferment galactose if they are grown in the presence of the sugar. Evidently, then, increase in cell number is sufficient to effect the physiological change in either population. The problem is, however, whether the change will occur without this mechanism.

The results on the test plates indicate that Db23B grown on glucose consists mainly of individuals that cannot ferment galactose. On this basis it would appear probable that such a population would have to depend on cell division to change its over-all character with respect to this property by increasing through selection the proportion of individuals that can ferment the sugar. Thus a population of this nature should not "adapt" in the presence of galactose if cell division were either completely or significantly depressed.

Such experiments were performed on Db23B. To keep multiplication down to a minimum the cells were washed in $m/15$ $KH_2PO_4$ and resuspended in this solution with purified galactose as the only addition. Under such conditions cell counts remain constant over long periods of time. Anaerobic gas production was followed by the usual manometric methods. So long as the total cell count remained constant no change was observed from the very low rate of (endogenous) $CO_2$ evolution. As pointed out previously, negative results such as these have little crucial interpretive value. They do, however, become more meaningful when they are combined with the test-plate data, and the fact that this strain (in common with many others cited in the literature) invariably adapts to the substrate when allowed to proliferate in its presence.

The results obtained with LK2G12 under the same conditions are entirely different. With this strain, adaptation occurs quickly and almost explosively as may be noted from figure 2. In this experiment exactly the same experimental conditions were imposed as obtained in the experiments on Db23B. As can be seen, a very sharp change in the physiological characteristics of the population took place at the end of about 3 hrs. During this period no measurable change in the total number of individuals occurred as determined by total counts at the beginning and end of the run. These are plotted on the same graph. We have here a clear cut case of "adaptation" without the formation of new cells. It may be mentioned that this lag period of 3 hrs. is under standard conditions, reproducible,

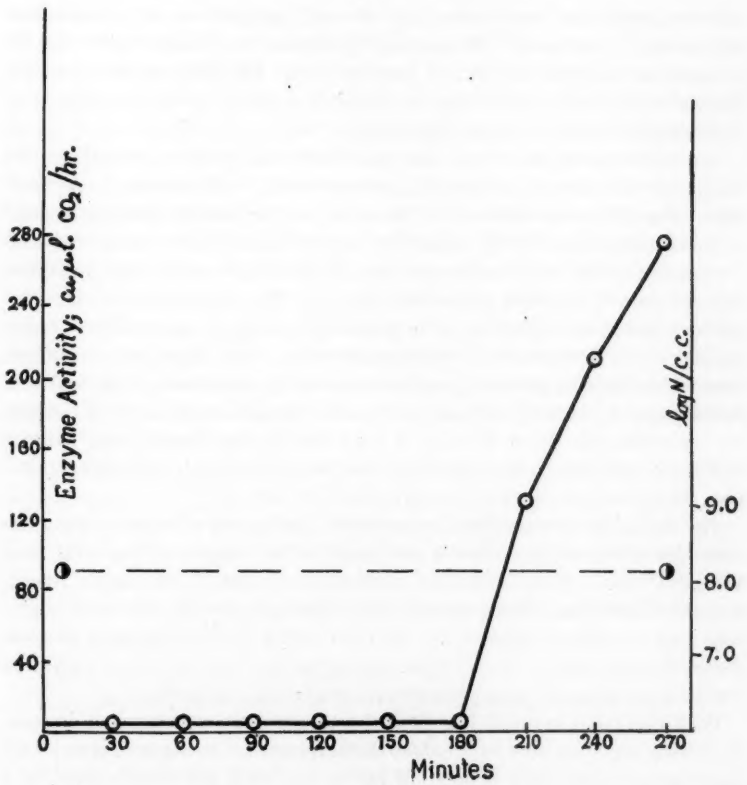


FIGURE 2

Change in physiological character of a stationary LK2G12 population in contact with galactose. ⊙ = Enzyme activity, ◑ = Log of number of cells per cubic centimeter.

and is characteristic for this strain. Other strains examined under the same conditions have longer lag periods extending up to 8 hrs.

*Discussion.*—These results are not only compatible with the previous test-plate examination of the phenotypic homogeneity of the populations but, in turn, the phenotypic pictures coincide well with what is known about the genetic background of the two strains employed; i.e., Db23B being principally composed of haploid cells would tend to be genetically unstable and consequently would give rise to phenotypically heterogeneous populations so long as vegetative reproduction is maintained.

These experiments offer a reasonable explanation for the apparently contradictory results obtained by various workers on the question of the rôle of

cell division in the "acclimatization" of yeast populations to galactose fermentation. Apparently Söhngen and Coolhaas,[4] as well as Euler[5] and his co-workers, were dealing with a culture of the Db23B type whereas Stephenson and Yudkin[6] probably employed a type represented by LK2G12, although with a much longer lag period.

The experiments described were specifically designed to investigate the biological mechanisms underlying populational "adaptations." As such they cannot provide solutions to the many biochemical problems of a more or less fundamental nature which have arisen during the course of the investigation. One important point may, however, be noted here which has as yet received too little attention; namely, that $CO_2$ evolution need not parallel galactose utilization as a source of energy for growth and maintenance. Of 34 strains of yeast examined in this laboratory, only one showed inability to grow in a medium containing galactose as sole source of carbohydrate, yet 80% of those that could use this sugar failed to ferment it. In particular the utilization of galactose by the "unadapted" culture of Db23B (glucose grown) was examined very carefully by following galactose disappearance during non-fermentative periods.

The existence of these non-fermentative oxidations of carbohydrate has been demonstrated in other forms using other sugars. Trautwein and Wiegend[13] have shown the direct oxidation of maltose by certain molds. Recently Barron and Freidemann[14] have demonstrated the oxidation of glucose and hexose-phosphates by bacteria which do not ferment glucose. As previously noted, a yeast strain possessing this type of glucose oxidation was isolated from one of our experimental glucose test plates.

With this point in mind, it is clear that the present investigation, in common with previous ones on "galactose adaptation," is not concerned with adaptation to galactose utilization *per se*, but with the development of a particular enzyme system leading to a fermentative oxidation of the sugar. The characteristics of the non-fermentative oxidations of galactose will be discussed elsewhere.

Whether it is appropriate to call the lag phenomenon observed with the LK2G12 strain in the presence of galactose an "adaptation," cannot be decided without an opportunity to examine the biochemical data in greater detail. Nor is it here possible to evaluate properly the usefulness of Karström's[10] classification of galactozymase as an "adaptive enzyme" in contradistinction to the glucose fermenting system as a "constitutive" one. Data obtained in this laboratory on the kinetics of the synthesis and destruction of the galactose fermenting system in a genetically stable population, would rather suggest that one was dealing with an enzyme system extremely unstable in the absence of its specific substrate and that what Karström calls a "constitutive" enzyme is simply one that is relatively more stable.

Whatever the case may be, the results reported here would suggest that a proper investigation into the nature of the acclimatization towards $CO_2$ production from galactose requires some knowledge of the genetic background of the strain being employed as well as an examination of the phenotypic homogeneity of the starting population. Unless this is done, there is the ever-present risk of unknowingly complicating the experiments by studying two distinct phenomena at the same time, one involving the competitive replacement of one phenotype by another and the other the kinetics of the enzyme substrate interaction. The latter problem can be studied safely only in a genetically stable and homogeneous population.

*Summary and Conclusions.*—1. A method is described involving the use of double-layered agar test plates which permits an examination of the phenotypic homogeneity of yeast populations with respect to the ability of individual members to produce gas from galactose. This method was used to elucidate the mechanism of enzymatic adaptation in a haploid and in a diploid strain of yeast.

2. Adaptation in the haploid was found to occur only in increasing populations.

3. Adaptation in the diploid could occur in the absence of cell division.

4. The necessity for a knowledge of the genetic background and phenotypic composition of initial populations is emphasized, to avoid complicating studies of enzyme synthesis with the kinetics of competitive interaction between phenotypes.

* Aided by a grant from Anheuser-Busch, Inc., St. Louis.

[1] Lippmann, E. V., *Die Chemie der Zuckerarten*, Braunschweig, 1904.

[2] Armstrong, E. F., *Proc. Roy. Soc.*, **B76**, 600–605 (1905).

[3] Slator, A., *Jour. Chem. Soc.*, **93**, 217–242 (1908).

[4] Söhngen, N. L., and Coolhaas, C., *Jour. Bact.*, **9**, 131 (1924).

[5] V. Euler, H., and Nilson, R., *Zeit. Physiol. Chem.*, **143**, 89–107 (1925).

[6] Stephenson, M., and Yudkin, J., *Jour. Biochem.*, **30**, 506–514 (1936).

[7] The word "new" is here used advisedly and is not meant to imply that the enzyme system formed was necessarily entirely foreign to the cell. Experimentally, due to the limitations of available techniques, it is impossible to distinguish between this situation and one where the enzyme was actually present but in too small an amount to be measured.

[8] Dubos, R. J., *Bact. Rev.*, **4**, 1–16 (1940).

[9] Yudkin, J., *Biol. Rev. Camb. Phil. Soc.*, **13**, 93–106 (1938).

[10] Karström, J., *Ergeb. Enzymforsch.*, **7**, 350–376 (1937).

[11] Shapiro, A., Spiegelman, S., and Koster, H., *Jour. Genetics*, **34**, 237–245 (1937).

[12] Lindegren, Carl C., and Lindegren, G., *Ann. Mo. Botan. Garden*, **30**, 453–468 (1943).

[13] Trautwein, K., and Wiegand, K., *Biochem. Z.*, **240**, 423 (1931).

[14] Barron, Guzman, E. S., and Friedemann, T. E., *J. Biol. Chem.*, **137**, 593–615 (1941).

## *ERRATA*

In the article, "On Convex Topological Linear Spaces," by GEORGE W. MACKEY, **29**, 315–319 (1943), in lines 22 and 23 the phrase "whose continuous linear functionals" should be replaced by "the pseudo-norm sets of whose continuous pseudo-norms."

In the article, "Natural Logarithms of Small Prime Numbers," by H. S. UHLER, **29**, 319–325 (1943), in line 2 up from the bottom of page 323 in the value of ln 43 the ninth pentad "12917" should be changed to read "12971."